USDA ARS National Animal Germplasm Program

White Blood Cell Cryopreservation Protocol

Sample collection:

Collect blood into purple top tubes containing EDTA as the anti-coagulant. Following collection, blood can be stored at 5 °C in purple top tubes for up to 7 days prior to processing. However, better white blood cell samples will be obtained if the holding time is minimized.

Centrifuge blood tubes at 800 x g for 20 min so that the sample is separated into plasma (top layer), buffy coat (middle layer; white blood cells), and erythrocytes (bottom layer; red blood cells).

Remove an aliquot of plasma (800 µL) and place it in a tube with 200 µL of dimethylsulfoxide.

Remove the buffy coat and dilute to 1 mL with neat plasma.

Combine the diluted buffy coat and the plasma/dimethylsulfoxide mixture and mix well.

Take care to minimize the amount of red blood cells present in the buffy coat samples.

Load the samples into 0.5 mL straws and cool to 5 °C.

Cryopreservation of the white blood cells is performed using a programmable freezer with the following freeze curve: 5 °C to -85 °C at 3.5 °C/minute.

Plunge the samples into liquid nitrogen for storage.

Thaw samples for 30 sec in a 37 °C water bath and process as necessary.

References:

Truax, R.E. et al., 1993. Cryopreservation of bovine buffy coat leukocytes for use in immunologic studies. Am J Vet Res 54:862-866.

Kleinschuster, S.J. et al., 1979. Cryopreservation of bovine monomuclear leukocytes. Am J Vet Res 1649-1651.

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